Spatial behavior in relation to mating systems: movement patterns, nearest-neighbor distances, and mating success in diploid and polyploid frog hybrids (Pelophylax esculentus)

Hoffmann, Alexandra ; Abt Tietje, Gaby ; Reyer, Heinz-Ulrich

Abstract: Mating success depends not only on genetic and phenotypic characteristics of males and females but also on their spatial position relative to other individuals, which influences the chances for interactions. Hence, any behavior that affects proximity to other individuals can potentially translate into fitness gain or loss. Here, we investigate the effects of genotype on male movement and distance to nearest neighbor (DNN) in three populations of the edible frog Pelophylax esculentus, a natural hybrid between its parental species Pelophylax ridibundus(genotype RR) and Pelophylax lessonae (LL). The system is particularly suited for such an analysis because the fitness differences between mating with a certain genotype are particularly strong. Moreover, which genotype should be preferred differs among populations where diploid hybrids (LR) live in sympatry withP. lessonae (L–E system) and those where diploid hybrids occur in allhybrid populations together with triploids (LLR and/or LRR) (E–E system). Hence, we expected differences among genotypes in movement patterns and spatial arrangement within the breeding pond. We did, indeed, find such differences. They were predominantly due to density differences between populations, followed by size and condition differences between males. Most relevant for our question was a difference in DNN: in the E–E system, distances between all three hybrid types were equal, whereas in the L–E system LR hybrids tended to stay closer to LL than to other LR. The results are discussed in relation to previous mate choice experiments and theoretical models about mating preferences in the two systems.

DOI: https://doi.org/10.1007/s00265-014-1862-0

Behav Ecol Sociobiol (2015) 69:501–517
DOI 10.1007/s00265-014-1862-0

ORIGINAL PAPER

Spatial behavior in relation to mating systems: movement patterns, nearest-neighbor distances, and mating success in diploid and polyploid frog hybrids (*Pelophylax esculentus*)

Alexandra Hoffmann · Gaby Abt Tietje · Heinz-Ulrich Reyer

Received: 11 August 2014 / Revised: 18 December 2014 / Accepted: 18 December 2014 / Published online: 7 January 2015

Abstract Mating success depends not only on genetic and phenotypic characteristics of males and females but also on their spatial position relative to other individuals, which influences the chances for interactions. Hence, any behavior that affects proximity to other individuals can potentially translate into fitness gain or loss. Here, we investigate the effects of genotype on male movement and distance to nearest neighbor (DNN) in three populations of the edible frog *Pelophylax esculentus*, a natural hybrid between its parental species *Pelophylax ridibundus* (genotype RR) and *Pelophylax lessonae* (LL). The system is particularly suited for such an analysis because the fitness differences between mating with a certain genotype are particularly strong. Moreover, which genotype should be preferred differs among populations where diploid hybrids (LR) live in sympatry with *P. lessonae* (L–E system) and those where diploid hybrids occur in all-hybrid populations together with triploids (LLR and/or LRR) (E–E system). Hence, we expected differences among genotypes in movement patterns and spatial arrangement within the breeding pond. We did, indeed, find such differences. They were predominantly due to density differences between populations, followed by size and condition differences between males. Most relevant for our question was a difference in DNN: in the E–E system, distances between all three hybrid types were equal, whereas in the L–E system LR hybrids tended to stay closer to LL than to other LR. The results are discussed in relation to previous mate choice experiments and theoretical models about mating preferences in the two systems.

Communicated by M. Gibbons

A. Hoffmann · G. Abt Tietje · H.-U. Reyer (✉)
Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
e-mail: uli.reyer@ieu.uzh.ch

Keywords Density · Hybridogenesis · Movement patterns · Mating system · Nearest neighbor · Water frogs

Introduction

In most populations, individuals are not evenly distributed and randomly assorted but according to some genetic and/or social structure. Such structure can arise passively as, for instance, in plants and sessile aquatic animals where the distribution of gametes and propagules is often determined by the range and direction of the dispersing agents, such as wind, currents, insects, and other animals (Grosberg 1987; Glaettli et al. 2006). In most animal species, however, individuals show active macro- and micro-habitat selection, i.e., they disperse to and settle in environments that are better than other areas in terms of climate, food, predators, diseases, and other abiotic and biotic environmental factors (Cody 1985; Orians and Wittenberger 1991; Sutherland 1996; Krebs 2009). As a result, different distributions of these environmental factors in space and time will translate into different spatial and temporal availability of potential mates and the ability to monopolize them. This, in turn, contributes to shaping sexual selection and can, to a large extent, explain the diversity of mating systems, social organizations, and patterns of parental care that we find among animals (Orians 1969; Emlen and Oring 1977).

However, mating opportunities and success not only depend on the distribution of ecological factors and its consequences for female and male distribution. The demographic and social environment individuals find themselves in also plays a role, i.e., density, age distribution and operational sex ratio, as well as relatedness and phenotypes of the conspecifics. Therefore, animals can actively modify their social environment by choosing the types and numbers of individuals that they prefer to interact with or want to avoid. Inferior

or less attractive males, for instance, may in some cases enhance their chances of obtaining a territory and/or a mate by avoiding the neighborhood of superior individuals, whereas in other cases they may improve their mating chances by staying as satellites close to superior males and benefitting from their high attractiveness to females (Arak 1983; Taborsky 1994; Cockburn et al. 2009; Oh and Badyaev 2010). In the latter case, there is a conflict between the best strategy for the inferior males seeking vicinity and the superior males that should avoid it. Hence, the optimal spatial and temporal distribution for the two parties will differ.

A typical situation where avoidance seems particularly necessary occurs when surrounding individuals are of a different species and hybrids are less fit (reviewed by Arnold 1997). Then, temporal and spatial segregation in breeding areas can help to reduce the frequency of interspecific interactions and, hence, the chances for mistakes or forced copulations. In some cases, however, the vicinity of heterospecifics should be looked for because mating with a different species may be advantageous under certain ecological conditions (Pfennig 2007; Reyer 2008) or even absolutely necessary for successful reproduction. The latter holds for taxa (most of them unisexual) in which females need the sperm of a heterospecific male to trigger the development of their eggs (gynogenesis) or regain a previously eliminated paternal genome (hybridogenesis) (Dawley 1989; Bullini 1994). In such situations, individuals of the gamete-dependent species should seek the vicinity of the heterospecific gamete donors, whereas individuals of the donor species should avoid the vicinity of and mating with the dependent species because the resulting offspring will not carry their genomes (gynogenesis) or discard them in the next generation (hybridogenesis). An excellent model system to study how this conflict of interests affects the movements and spatial distribution of the participants is provided by the subject of this study, the European water frog *Pelophylax esculentus*.

The Palaearctic water frog complex (*Pelophylax*)

Pelophylax esculentus is a natural hybrid between the pool frog *P. lessonae* (genotype LL) and the marsh frog, *P. ridibundus* (genotype RR). *P. esculentus* reproduces through hybridogenesis (Tunner 1974), a hemiclonal reproductive mode first described in fishes of the genus *Poeciliopsis* by Schultz (1969): hybridogenetic organisms exclude the genome of one parental species prior to meiosis and transfer the genome of the other parental species clonally to the next generation, i.e., without recombination (Tunner 1974; Tunner and Heppich-Tunner 1991; Zalesna et al. 2011). In order to restore hybridity in their offspring, hybrid males and females have to live in sympatry and back-cross with the parental species whose genome is excluded (Uzzell and Berger 1975), i.e., *P. lessonae* when the L genome is excluded (L–E system) and *P. ridibundus* when the R genome is excluded (R–E system) (Graf and Polls Pelaz 1989). In both systems, the hybrid is a sexual parasite that relies on a parental species (the sexual host) for successful reproduction, and in both systems, the resulting offspring are predominantly diploid *P. esculentus* (genotype LR). Although triploid hybrid forms can occur in these two systems, populations containing them appear to be restricted to certain geographical areas in Europe (Hoffmann et al., submitted). The vast majority of the sexually parasitic system consists of one of the two parental species and diploid LR hybrids.

A different situation exists in all-hybrid populations consisting of diploid (LR) and triploid hybrids (LLR and/or LRR). These so-called E–E systems occur mainly in northern Europe around the Baltic Sea, but also in some areas of eastern and Central Europe. Here, hybrids have become reproductively independent of the parental species as a result of polyploidization and "meiotic hybridogenesis" (Alves et al. 2001). This means that triploids of both sexes premeiotically exclude the genome present in one copy (R in LLR and L in LRR), recombine the double genome, and transfer it to haploid gametes (L in LLR and R in LRR)—as the parental species *P. lessonae* and *P. ridibundus* do in L–E and R–E systems, respectively. When these haploid gametes fuse with diploid ones (LR) that are usually produced by diploid females, triploid offspring result; when they fuse with heterospecific haploid gametes produced by other triploids or by diploid LR, usually males, diploid offspring arise (Günther et al. 1979; Christiansen et al. 2005; Arioli 2007; Christiansen 2009; Christiansen and Reyer 2009). As a result, both hybrid types are mutually dependent on each other: triploids are the sexual hosts for diploid sexual parasites and vice versa (Som and Reyer 2006).

According to these scenarios, hybrids in diploid L–E and R–E systems should have evolved a preference for mating with individuals of the parental species because these are always the mates to be preferred. In contrast, genetic fixation of mate preferences in a particular genotype should be impossible in diploid–triploid all-hybrid populations because here the to-be preferred mating partners alternate each generation: diploid LR females producing diploid eggs should choose triploid LLR or LRR males; the resulting triploid daughters should choose diploid LR males, etc. For females from L–E and E–E systems, these predictions from theoretical models (Hellriegel and Reyer 2000; Som et al. 2000; Som and Reyer 2006) have, indeed, been supported both in natural populations (Blankenhorn 1974; Günther and Plötner 1990; Lengagne et al. 2006, 2008; Lengagne and Joly 2010; GAT, unpublished data) and in experimental settings (Abt and Reyer 1993; Reyer et al. 1999; Roesli and Reyer 2000; Engeler and Reyer 2001; Rondinelli 2006). For R–E systems, we do not

know of any detailed study on mate choice and mating behavior; therefore, we will not refer to R–E systems for the rest of this paper.

The two parental species differ in several features, including habitat preferences, morphology, physiology, and mating behavior (for reviews, see Günther 1990; Plötner 2005). Relevant for the present study are differences in space use during the reproductive season: *P. lessonae* males tend to roam around within large choruses to intercept and clasp females, while *P. ridibundus* males show a tendency for site tenure (Blankenhorn 1974; Tunner 1976; Lengagne et al. 2008) and even defense of small areas that provide access to females and/or to resources used by females, such as oviposition sites (Kuhn and Schneider 1984; Kyriakopoulou-Sklavounou and Loumbourdis 1990).

Hybrids take an intermediate position between the two parental species for many characteristics, including morphology and male vocalization (e.g., Kierzkowski et al. 2011; Hoffmann and Reyer 2013), and they occupy a broader spectrum of biotic and abiotic habitat conditions than the parental species (Plenet et al. 2000; Pagano et al. 2001; Holenweg Peter et al. 2002). In terms of mating behavior, *P. esculentus* males seem to share the tendency to be stationary at territories with *P. ridibundus* (Weidenberg 1999) but are also active in intercepting and clasping females like *P. lessonae* (Lengagne et al. 2006, 2008). In all-hybrid E–E systems, the intermediate position of *P. esculentus* has been investigated on an even finer scale, namely, with respect to a genome dosage effect. Given that the ratio of the parental L/R genomes decreases from 2:1 in triploid LLR hybrids through 1:1 in diploid LR to 1:2 in triploid LRR, we can expect LLR to resemble more *P. lessonae*, while LRR should resemble more *P. ridibundus.* This has indeed been found for habitat preferences (Jakob et al. 2010), phenotypic characteristics like body size and morphology (Ebendal 1979; Ebendal and Uzzell 1982; Plötner et al. 1994; Kierzkowski et al. 2011; Embrechts and Reyer 2012), and recently also for male advertisement calls (Hoffmann and Reyer 2013). However, dosage effects are not always pronounced and could not be detected in some populations and for some phenotypic, biochemical, and acoustical parameters (Plötner and Klinghardt 1992; Tunner 2000; Hoffmann and Reyer 2013).

In the present study, we examined whether the reproductive strategies of *P. esculentus* in terms of space use (roaming versus site fidelity) also differ between the three hybrid genotypes (LLR, LR, LRR) in line with a genomic dosage effect and, if so, whether such differences translate into different reproductive success. We expected that triploids should behaviorally tend more towards the parental species they share two genomes with, i.e., LLR males should move around more, LRR males should be more stationary, and LR individuals should be intermediate between the two types of triploids. Specifically, we addressed the following questions:

1. Do the distances males move between observations and the distribution of their overall home ranges differ between individuals of different body size, body condition, and/or genotype?
2. Do nearest-neighbor distances vary between genotypes?
3. Is the frequency of amplexus events affected by composition of the population and/or by male characteristics like space use, body size, body condition, and genotype?

To answer these questions, we compared the reproductive success and spatial behavior of genotypes during the reproductive period. Reproductive success was estimated by amplexus frequency and space use by regularly recording the positions of marked individuals. As an indicator of spatial tenure, we measured the distance an individual male had moved between subsequent transect observations and the distance to the center of its home range. To account for potential population differences, especially those between L–E systems with and E–E systems without mate preferences (see above), we performed our study in three different ponds, one in Switzerland with an L–E population and two in Germany and Sweden with E–E populations.

Methods

Sampling periods and sites

Field data for this study were collected at three natural ponds located in Northern Switzerland (Kloten), Eastern Germany (Döbern), and Southern Sweden (Genarp). These three ponds were situated more than 500 km apart (Fig. 1). Catching, marking, and transect sampling took place during the periods shown in Table 1. In Kloten, where data were originally collected in the scope of a different study (Abt Tietje 2003), the field work was performed until the end of August. For the present study, however, only data from May and June 1992 were used to cover the same seasonal period for all three ponds.

Capturing, measuring, and marking of frogs

At all three ponds, frogs were captured by hand at night using flashlights and kept in cool and moist boxes until the next morning. Then, they were measured (snout–vent length, SVL) to the nearest millimeter using calipers and weighed to the nearest 0.5 g with a spring balance. From SVL and body mass (BM), we later calculated a body condition index (BCI) according to the equation $BCI = BM/SVL^3$ (Jakob et al. 1996). After measuring, each frog was marked with an individually numbered tag that was fitted around the frog's waist with dental floss (1992) and rubber thread (2009), respectively. The bands were fitted loosely enough to allow for normal

Fig. 1 Map showing the localities of the three ponds: Kloten (Switzerland), Döbern (Germany) and Genarp (Sweden)

movement, including oviposition in females. In 1992, we recaught the frogs at the end of the season and removed the waistbands, whereas the thread used in 2009 was degradable and fell off by itself after the observation period. We marked

Table 1 Sampling and pond details for the three studied populations. In addition to the above-water vegetation listed under pond structure, there was in Genarp a coherent patch of submerged vegetation (about 10 % of the total pond area) that reached close to the surface (see Fig. 2)

		Kloten	Döbern	Genarp
Sampling period		15 May–20 June 1992	24 May–2 June 2009	13–26 June 2009
Total number of observations		37	14	12
Water temperature		12–20 °C	12–25 °C	10–20 °C
Pond structure		Open water 45 %	Open water 25 %	Open water 80 %
		Water lilies 25 %	Water lilies 20 %	Reeds 12 %
		Sedge 30 %	Reeds 35 %	Sedge 8 %
			Two islands 20 %	
Pond size (m^2)		90	537	3352
Population size		~350	~260	~560
Density (frogs/m^2)		3.9	0.48	0.15
Sex ratio (M/F)		0.85	1.44	2.01
Genotype composition (%)	LL	68.1		
	LR	31.9	33.8	94.4
	LLR		26.5	
	LRR		39.7	5.6

only adult frogs >45 mm that could be reliably sexed by the presence (males) or absence (females) of vocal sacs and thumb pads.

For determining genotypes, we took lymph from a small incision into the foot web (1992), respectively, two toe clips from the 1st phalanx of the 2nd digit of the front legs (2009). The clips were stored in 70 % ethanol, and from the wound a blood smear was produced on a microscopic slide. As taking toe clips and blood smears for genotype determination was more invasive than taking lymph, we attempted to minimize stress as much as possible. Therefore, all frogs sampled in 2009 were anesthetized before handling by bathing them for 10–30 min in a diluted and buffered MS222 solution (Mitchell 2009). After handling, they were kept in a moist and cool lidded box and given 2–3 h to recover before they were released back into the pond. Thereafter, they were observed for a few minutes to make sure they resumed normal activity, which all of them did (e.g., hopping directly towards the water, swimming towards floating vegetation, and calling). We did not observe any physical impairment or deaths as a consequence of handling in any of the three ponds.

Genotype determination

In 1992, genotypes in the Kloten population were determined through protein electrophoresis of lymph samples, following standard procedures (e.g., Uzzell and Berger 1975; Uzzell and Hotz 1979). In 2009, we performed microsatellite analysis on DNA extracted from the toe clips, which reliably separated the three genotypes. Of the 16 markers that we used, four are specific for the L genome and eight for the R, and four amplify in both genomes with a dosage effect. This marker combination allows unambiguous identification of all genotypes, including distinction between LLR and LRR hybrids. Details of the markers and the lab techniques have been described elsewhere (Christiansen 2005, 2009; Christiansen and Reyer 2009; Arioli et al. 2010; Jakob et al. 2010).

Pond features and population density

We surveyed and sketched the ponds true to scale, including details on the type and extent of the vegetation. In 1992, we measured the Kloten pond size from this sketch, whereas in 2009 the pond areas for Döbern and Genarp were obtained from aerial pictures using the measuring feature in program Quantum GIS (QGIS Development Team 2013). Pond sizes were rounded to the nearest square meter. To estimate population size and density, different methods had to be used for the 2 years of the study. In 1992, in Kloten we recorded only marked individuals during our daily observations (see below), but we caught animals repeatedly through the whole season. This capture–mark–recapture design allowed calculation of the population size through the Jolly–Seber method (Caughley 1980). In contrast, in 2009, frogs in the Döbern and Genarp ponds were, once marked, not captured again, except if they had lost their tag. Thus, the assumption of the Jolly–Seber method, that marked and unmarked animals are equally vulnerable to capture, was not met in these populations. The capture data from Döbern and Genarp corresponded more to a depletion capture method since we captured and marked as many frogs as possible at the beginning of the season during repeated captures. The assumptions of this method (e.g., relatively small capture area, negligible amounts of emigration during the sampling period, all frogs equally vulnerable to capture) were met in both ponds. We thus used the capture data from Döbern and Genarp to stimate

population size based on depletion capture counts as described by Zippin (1958). Densities were calculated by dividing the estimated population sizes by the respective pond size.

Data collection

Data were collected by slowly walking along the edge of a pond, recording locations of the frogs, and entering them into a detailed map of the pond. Positions could be determined with a precision of 10–20 cm through a combination of prominent vegetation in the pond, natural features of its bank, and sticks that we had placed as artificial landmarks along its edge. In Kloten, we recorded only marked individuals; in Döbern and Genarp, unmarked frogs were recorded as well, but genotypes (and sometimes sexes) of the unmarked ones were not securely distinguishable. In accordance with previous studies on hybrid *P. esculentus*, frogs <45 mm in body length and with no perceptible sexual characteristics were recorded as "juvenile" (Arioli 2007; Christiansen and Reyer 2009). In all three ponds, marked males in amplexus were documented whenever observed. If an amplexus pair was encountered and the male was not marked, we tried to capture the male to determine its genotype, measure its size and weight, and mark it individually for later observation.

With the exception of cold and rainy days, when there was no calling activity, observation rounds were done daily one to three times (also depending on weather) during daytime over the periods given in Table 1. To guarantee independence of the data, we allowed for a minimum of 5 h between successive observations. Since the mating activity of frogs is strongly temperature dependent, we measured water temperature continuously. In Döbern and Genarp, we placed three thermocouples each 15 cm below the surface, recorded and stored temperatures every 30 min on loggers and calculated means across the three loggers. In Kloten, water temperature was not measured in 1992, but continuous logger recordings from 1994 to 1996 in the same pond showed that in all 3 years, water temperatures during peak reproductive activities were very similar. Moreover, average monthly air temperatures measured at the nearby Kloten station were almost identical during the time of breeding activities: in 1992, they ranged from 8.4 in April to 16.2 °C in June and in 1994–1996 from 8.6 to 16.4 °C for the same months (IDAWEB, MeteoSwiss). Therefore, we believe that the 1994–1996 water temperatures are also representative for those in 1992.

Calculation of spatial data

The observation sheets with the frog locations were digitized with geographical information system (GIS) software (Quantum GIS in 2009). Coordinates of all recorded frogs were saved as point vector layers. These were imported into spreadsheet format to sort the data by individuals and link them with genotype, sex, size, weight, and body condition. From the location data of marked individuals, we derived three spatial measurements:

1. Distance between observations (DBO): For each individual that was observed more than three times, we calculated the Euclidean distance between two subsequent observations. These distance measurements were then averaged across the number of total observations per individual to get a measure for the spatial activity.
2. Distance to center (DTC): For each individual with more than three observations, the distance of each recorded location to the center of the activity range (=centroid) was calculated. Although to some extent correlated with DBO, this variable provides additional information whether a frog's movements were condensed in a certain area or spread out over a larger area. Hence, it is an approximation of site fidelity.
3. Distance to nearest neighbor (DNN): To measure proximity to the nearest neighbor, we averaged the distance of a given male to its closest male neighbor across all occasions the focal individual was observed (minimum of three observations).

In the field, we did not observe any physical interactions between males that were more than 3 m apart, and only 2.8 % of 1265 nearest-neighbor distances were larger than 3 m. Therefore, we considered only nearest-neighbor distances of ≤3 m relevant for physical male–male interactions. Most of the nearest-male distances >3 m were measured in Genarp and Kloten. For Genarp, we assume that the larger DNN values (max. 19 m) can be attributed to the generally low population density, while the Kloten outliers may have resulted from the less stringent sampling regime which did not account for unmarked individuals. We thus felt confident that omitting all cases of distances >3 m between males provides a justified restriction of the data set for further analysis. Since we tested for the influence of genotype and morphological characteristics on nearest-neighbor distance, we only considered cases where the genotype and body measurements of both neighbors were known. During an observation round, each individual neighbor pairing was only recorded once, and thus pseudoreplication was avoided.

Statistical analysis

All statistical tests were performed using the program Systat (version 11). To compare the three distance measures DBO, DTC, and DNN between and within populations, genotypes, and sexes and to relate them to body size and condition, we performed generalized linear models (GLM). In the analysis of DNN, we incorporated body size and condition as (a) the

averaged values between the two nearest neighbors (AV_SVL and AV_BCI) and (b) the difference between these two values (DIFF_SVL and DIFF_BCI). Unless otherwise stated, probability levels below α=0.05 were considered significant. For post hoc tests, we used Fisher's least significant difference test with subsequent Bonferroni correction. When plotting centroid coordinates of individuals from a pond onto a map, we calculated kernel density estimates using the built-in function in Systat and choosing the default probability level of 68 %. The observed distribution of genotypes among males in amplexus was tested against the expected distribution of genotypes among males in the population using chi-square tests.

Results

Pond features, population composition, and frog characteristics

All three ponds were exposed to full sun, except during the very early and late hours of the day when surrounding bushes and small trees casted some shade. As a result, water temperature ranges were also very similar (Table 1). Temperatures >15 °C occurred in all three ponds, and thus conditions were favorable for mating activities (Wahl 1969; Blankenhorn 1974; Heym 1974)—although lower temperatures occasionally existed due to fluctuations between day and night and periods of warmer versus colder weather. In several other features, however, the ponds differed, including surface area, percentage of open water, vegetation below and above the surface, frog density, composition of the population in terms of genotypes and sex ratio, and frog size (SVL) and body condition index (BCI) (for details, see Table 1 and Fig. 2). Frog density per square meter decreased from Kloten, the smallest pond, through Döbern to Genarp, the largest pond, while the overall male/female ratio increased in the same direction. In terms of demographic composition, the Kloten population was an L–E system with an LL/LR-ratio of 6.93 among males and 1.06 among females. The Döbern and Genarp populations were both E–E systems but differed in their genotype composition. In Döbern, most hybrids were triploid and occurred in both forms (LLR, LRR), whereas in Genarp the majority was diploid, and only LRR triploids were found (Table 1). Among males, we sampled 60 of the LR (Kloten 14, Döbern 15, Genarp 31), 17 of the LLR (Kloten 0, Döbern 17, Genarp 0), 26 of the LRR (Kloten 0, Döbern 25, Genarp 1), and 97 of the LL genotype (Kloten 97, Döbern 0, Genarp 0). Among females, we sampled 70 LR (Kloten 63, Döbern 6, Genarp 1), 2 LLR (Kloten 0, Döbern 2, Genarp 0), 6 LRR (Kloten 0, Döbern 5, Genarp 1), and 67 LL individuals (Kloten 67, Döbern 0, Genarp 0).

According to two GLM analyses—which also included genotype, sex, and their interaction—frog phenotypes also differed between populations (Table 2; Fig. 3). Body size (SVL) averaged across all genotypes within a population was largest in Döbern (70.02±6.89 mm (mean ± 1 S.D.)), second largest in Genarp (63.89±5.93 mm), and smallest in Kloten (53.07±7.57 mm; $p \leq 0.006$ for all pairwise post hoc comparisons, Bonferroni-corrected α=0.0167). Among males, body size of LL individuals (49.88±2.90 mm) was smallest of all groups, whereas body size among the three genotypes of hybrid males showed some overlap (LR 63.20± 6.28 mm, LLR 66.78±3.37 mm, LRR 71.91±5.06 mm), with a tendency of LRR males to be larger than LLR and LR males (p-values of pairwise comparisons are shown in Table 3). Females were, on average, larger than males (LL 50.94± 4.62 mm, LR 61.12±10.46 mm, LLR 81.51±0.69 mm, LRR 73.03±15.23 mm; p<0.0001; Table 2). With respect to genotype, only size differences between LL (males and females) versus most other genotypes of both sexes were significant (Table 3). There was, however, a significant sex*genotype interaction for SVL. While LL, LR, and LRR females were only slightly (2–3 %) and, at most, marginally significantly larger than their conspecific males (all $p \geq 0.059$), they were markedly larger in LLR (24 %; p=0.002; Table 3).

In terms of the body condition index (BCI), only pairwise differences between Kloten (1.02±0.13 units (mean ± 1 S.D.), and Döbern (0.95±0.11 units), and between Kloten and Genarp (0.94±0.08 units) were significant (Kloten–Döbern p=0.017, Kloten–Genarp p<0.0001, Döbern–Genarp p= 0.073; Bonferroni-corrected α=0.0167; Fig. 2b). For BCI, we did not find an interaction between sex and genotype. Rather, differences between the sexes were significant across all genotypes with females showing average BCI values of 0.04 units lower than males (p=0.008; Table 2). According to pairwise genotype comparisons, BCI differed significantly between LR and LRR (p=0.013, Bonferroni-corrected α= 0.0125), but not among the rest of genotypes (Fig. 3b).

Spatial distribution and movements in relation to frog traits and pond features (question 1)

Results from two stepwise GLMs revealed a highly significant influence of population on both the mean distance between two subsequent observations of the same frog (DBO) and the distance to the center of its movement range (DTC) (Table 4). For both variables, values were larger in Genarp than in the other two populations, which did not differ (Fig. 4a, b). Sex, genotype, size, body condition, and the interaction between sex and genotype, which were also included in the GLM analyses, did not contribute to either the model for DBO nor DTC, although for DBO there was a tendency for females to move slightly more than males (Table 4).

Concerning the location of the centroids, there was no indication of any sex- or genotype-specific spatial structuring. In Kloten, all four areas of the 68 % kernel estimates covered

mainly the right half and the center part of the pond (Fig. 5). The left side of the pond was less frequented by the frogs, probably due to a coherent patch of dense sedges in this area (see Fig. 2). In Döbern, we also found great overlap among the movement centroids of LLR, LR, and LRR, both for males (Fig. 6a–c) and females (Fig. 6d). According to these graphs, several centroids are located in areas that overlap with the two islands shown in Fig. 2. This does not mean that frogs actually moved around on or travelled terrestrially across these islands. We only observed frogs on land by the very edges of the

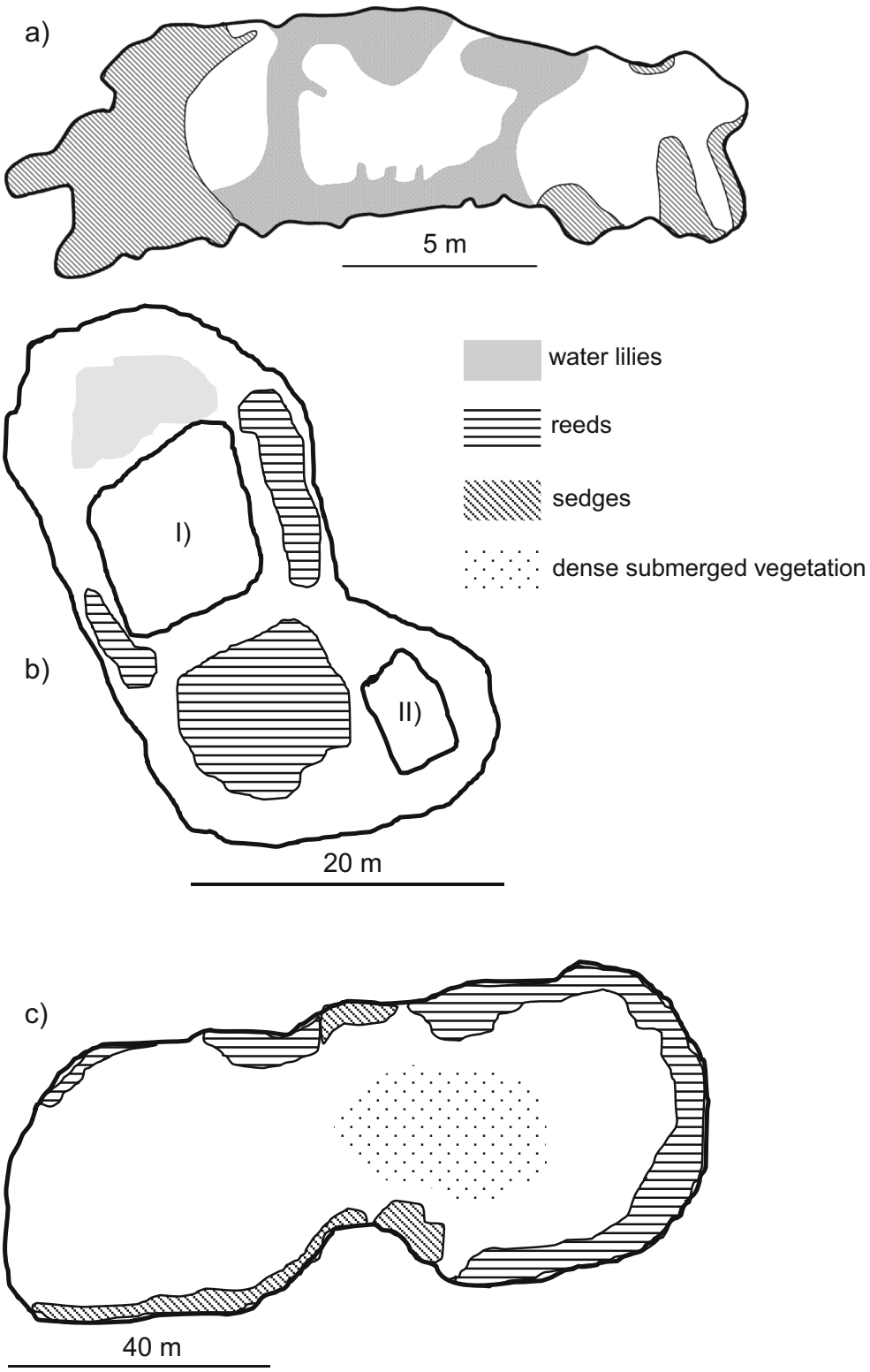


Fig. 2 Schematic illustration of the three study ponds, **a** Kloten, **b** Döbern, and **c** Genarp, with distribution of open water and predominant vegetation types. *I* and *II* in **b** indicate vegetated islands in the Döbern pond (for further details, see Table 1)

Table 2 GLM of body size (SVL) and body condition index (BCI) versus population, genotype, and sex (significant *p*-values are in italics)

Source	SVL			BCI		
	df	*F*-ratio	*p*	df	*F*-ratio	*p*
Population	2	20.477	*<0.0001*	2	11.166	*<0.0001*
Genotype	3	24.910	*<0.0001*	3	4.001	*0.008*
Sex	1	12.546	*<0.0001*	1	7.035	*0.008*
Sex * genotype	3	2.917	*0.034*	3	0.677	0.566
Error	335			335		

islands; but recordings on opposite sides will inevitably result in centroids lying on the island. The island edges were attractive spots for basking. Especially LRR males frequented them, which explains why their centroids are concentrated in the island locations. In Genarp, only LR males could be tracked in sufficient numbers. This main distribution of their movement centroids is located in the center of the pond where the two banks come closest and a large and dense patch of submerged vegetation reaches close to the water surface. While the banks and vegetated edges of the pond were used for basking and occasional calling, this patch of submerged water vegetation was a preferred spot for mating. Amplexus pairs moved there for spawning, and during times of high calling activity, males frequented the area in search of unpaired females.

Spatial distribution in relation to other individuals (question 2)

Since the distribution of centroid localities showed that males were attracted to and aggregated in certain areas of the pond, we examined the nearest-neighbor distances between males to investigate the small-scale patterns of their spatial distribution. For the reasons explained under "Methods", we only considered distances <3 m in the analyses. Within this radius, the average distance males kept to their nearest male neighbor was highest in Genarp, followed by Kloten and Döbern (Fig. 7). Two GLMs showed that genotypes of the neighboring males did not affect nearest-neighbor distances in the mixed ploidy E–E system of Döbern but tended to do so in the diploid L–E system of Kloten (Table 5). Here, the average distance between hybrids (LR–LR) was higher than average distances between the other two combinations (LL–LL and LL–LR) (Table 5; Fig. 7). The two analyses further revealed that in Kloten distances significantly decreased with the average body size (AV_SVL) and body condition (AV_BCI) of the neighbors, whereas in Döbern distances decreased with the difference in body condition between them (DIFF_BCI). In Genarp, where the genotype effect could not be tested due to the almost exclusive occurrence of LR–LR neighborhoods, we tested for potential effects of body size and condition by means of multiple regression but did not find any effect on nearest-neighbor distances (Table 5).

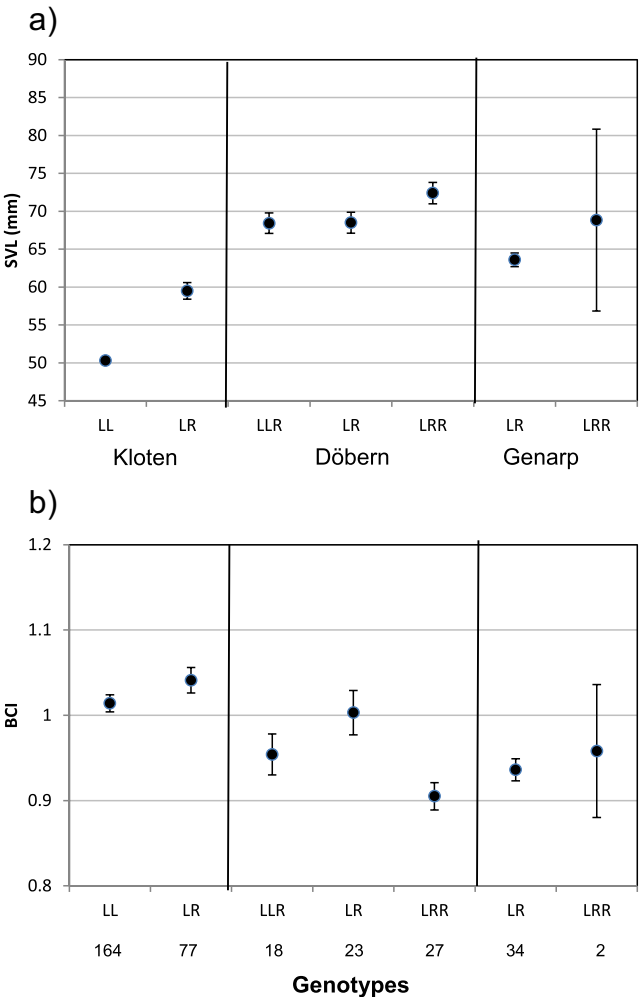


Fig. 3 Male body characteristics by genotype for the three ponds. *SVL* (snout–vent length) in **a** represents male body size (in mm). *BCI* in **b** means body condition index and was calculated from body size and weight (see "Methods"). *Symbols* and *error bars* indicate group means ± 1 SE. *Numbers below the X-axis* in **b** show genotype-specific sample sizes for both SVL and BCI

Reproductive behavior (question 3)

From a total of 51 amplexus pairs (Kloten 15, Döbern 22, Genarp 14), we determined the genotypes of the involved males and compared their relative frequencies with the overall distribution of male genotypes in the population. In the two all-hybrid E–E populations of Döbern and Genarp, the relative frequencies of amplexed male types did not differ from the overall distribution of the three genotypes within the population (Döbern χ^2=1.47, p=0.48, df=2; Genarp: χ^2=0.61, p=0.74, df=2). In the L–E population from Kloten, however, there was a tendency for *P. lessonae* males to be disproportionally more successful in clasping females into amplexus than *P. esculentus* males (χ^2=3.131, p=0.077,

Table 3 Matrix of pairwise comparison probabilities for SVL differences after Fisher's least significant difference test. The *p*-values which are significant after Bonferroni correction (=0.00625) or tend to be so are printed in bold and italics, respectively

	LL-F	LL-M	LLR-F	LLR-M	LR-F	LR-M	LRR-F	LRR-M
LL-F	1.000							
LL-M	0.2810	1.000						
LLR-F	**<0.0001**	**<0.0001**	1.000					
LLR-M	**0.024**	**0.006**	**0.002**	1.000				
LR-F	**<0.0001**	**<0.0001**	0.018	0.103	1.000			
LR-M	**<0.0001**	**<0.0001**	**0.003**	0.628	*0.059*	1.000		
LRR-F	**<0.0001**	**<0.0001**	0.125	0.019	0.265	0.030	1.000	
LRR-M	**<0.0001**	**<0.0001**	0.039	*0.008*	0.425	*0.015*	0.561	1.000

df=1). We had measurements of distance variables for 27 of the 51 amplexed males (Kloten 8 out of 15, Döbern 12 out of 22, Genarp 7 out of 14). Logit regressions on this subsample of males, separately for Kloten, Döbern, and Genarp, revealed no effect of the considered independent variables DTC, DBO, SVL, and BCI on amplexus success in males in any of the populations (Kloten: DTC all $p \geq 0.190$, DBO all $p \geq 0.205$; Döbern: DTC all $p \geq 0.134$, DBO all $p \geq 0.507$; Genarp: DTC all $p \geq 0.380$, DBO all $p \geq 0.415$).

Discussion

Among the variables that we tested, the effects on movement and spatial distribution of water frogs decreased from study population through body size to body condition and genotype. Study population influenced all three distance measures: between observations (DBO) to centroid (DTC) and to nearest neighbor (DNN). Body size and condition affected DNN in two populations, and genotype had only a marginal effect on DNN in one population. Below, we first discuss these results one after the other and then provide some general conclusions.

Table 4 GLM of movement distance parameters (DBO and DTC) versus population, genotype, and sex (significant *p*-values are in italics)

Source	DBO			DTC		
	df	*F*-ratio	*p*	df	*F*-ratio	*p*
Population	2	254.170	*<0.0001*	2	74.100	*<0.0001*
Sex	1	3.421	0.065	1	0.718	0.397
Genotype	3	1.927	0.125	3	0.356	0.785
SVL	1	0.448	0.504	1	0.062	0.804
BCI	1	0.193	0.660	1	0.354	0.552
Sex * genotype	3	0.940	0.422	3	0.193	0.901
Error	278			278		

Spatial behavior in relation to population, body size, condition, and genotype

Study population The three ponds showed considerable differences in size and population density: Kloten, the smallest pond, had the highest frog density and Genarp, the largest pond, the lowest one; Döbern was intermediate in both pond size and density. Distance parameters between Kloten and Döbern were almost identical, whereas individuals in Genarp moved significantly longer distances between subsequent observations (DBO), covered larger ranges, as indicated by larger distances to the centroid (DTC), and kept larger nearest-neighbor distances (DNN) than in the other two ponds.

One potential explanation for these pond differences is that increasing density automatically reduces distances, as more and more frogs have to partition the available space among them. This, however, cannot be the only reason because density in Döbern was more similar to Genarp (Table 1), but distances were very similar to Kloten (Fig. 4). However, what matters may not be density relative to overall pond size but relative to the area of preferred locations. In all three ponds, certain areas were more frequented than others. Males and females of all genotypes seemed to have the same preferences because the 68 % kernel areas of their movement centroids overlapped (cf. Figs. 2, 5, and 6). These preferred areas included structures like pond banks and vegetation types suitable for sun basking, hiding, ovipositing, and encountering sexual partners. Unfortunately, the size of these attractive areas was impossible to measure; but the smaller they are, the more densely males should aggregate in them. As a consequence, movements and nearest-neighbor distance will be reduced, even when density relative to pond size is low.

Another factor responsible for distance differences between ponds could be the sex ratio. While variation in population density affects the availability of mating partners for both sexes, this effect can be amplified for one sex in case of a skewed operational sex ratio (Kokko and Rankin 2006). Male–male competition was probably more intense in Genarp,

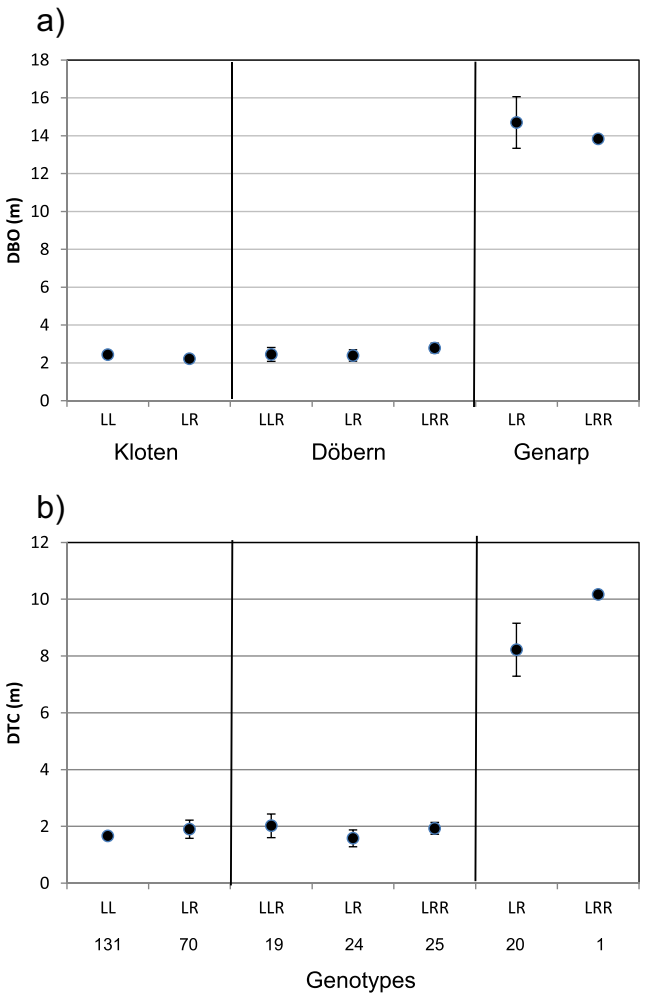


Fig. 4 **a**, **b** Distance measurements by population and genotype. *Symbols* and *error bars* indicate group means ± 1 SE. *Numbers below the X-axis* in **b** show genotype-specific sample sizes for both DBO and DTC

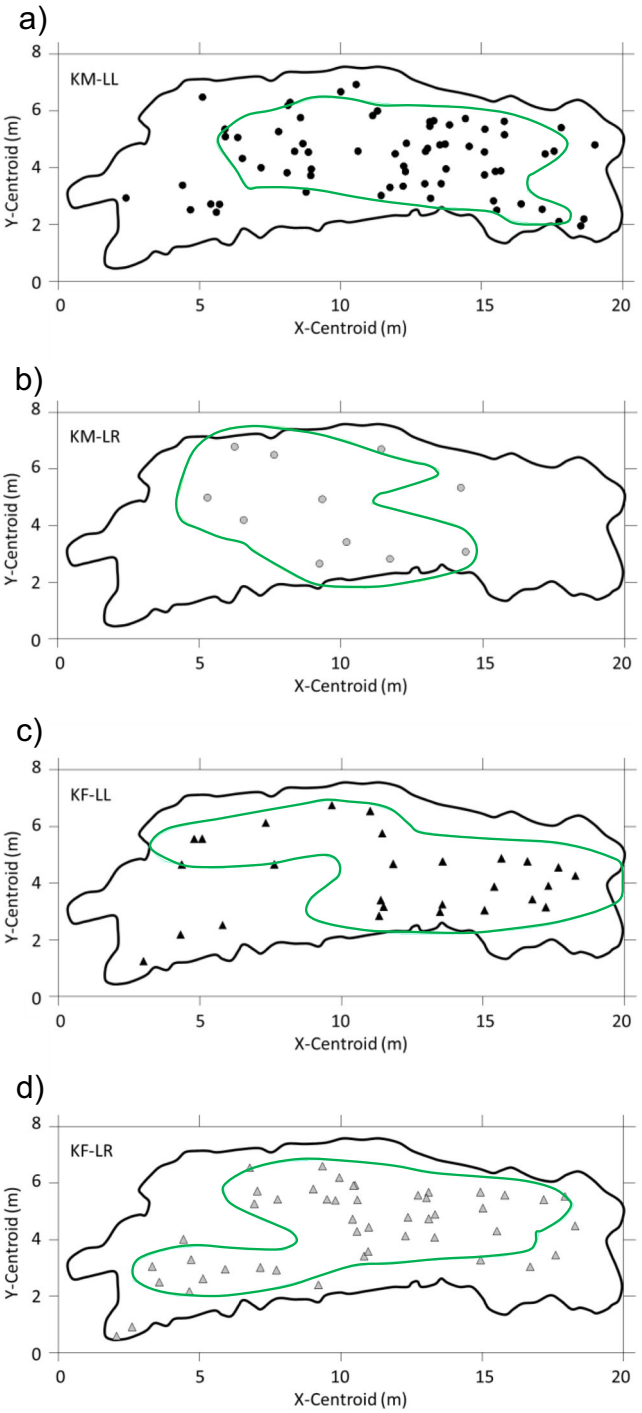
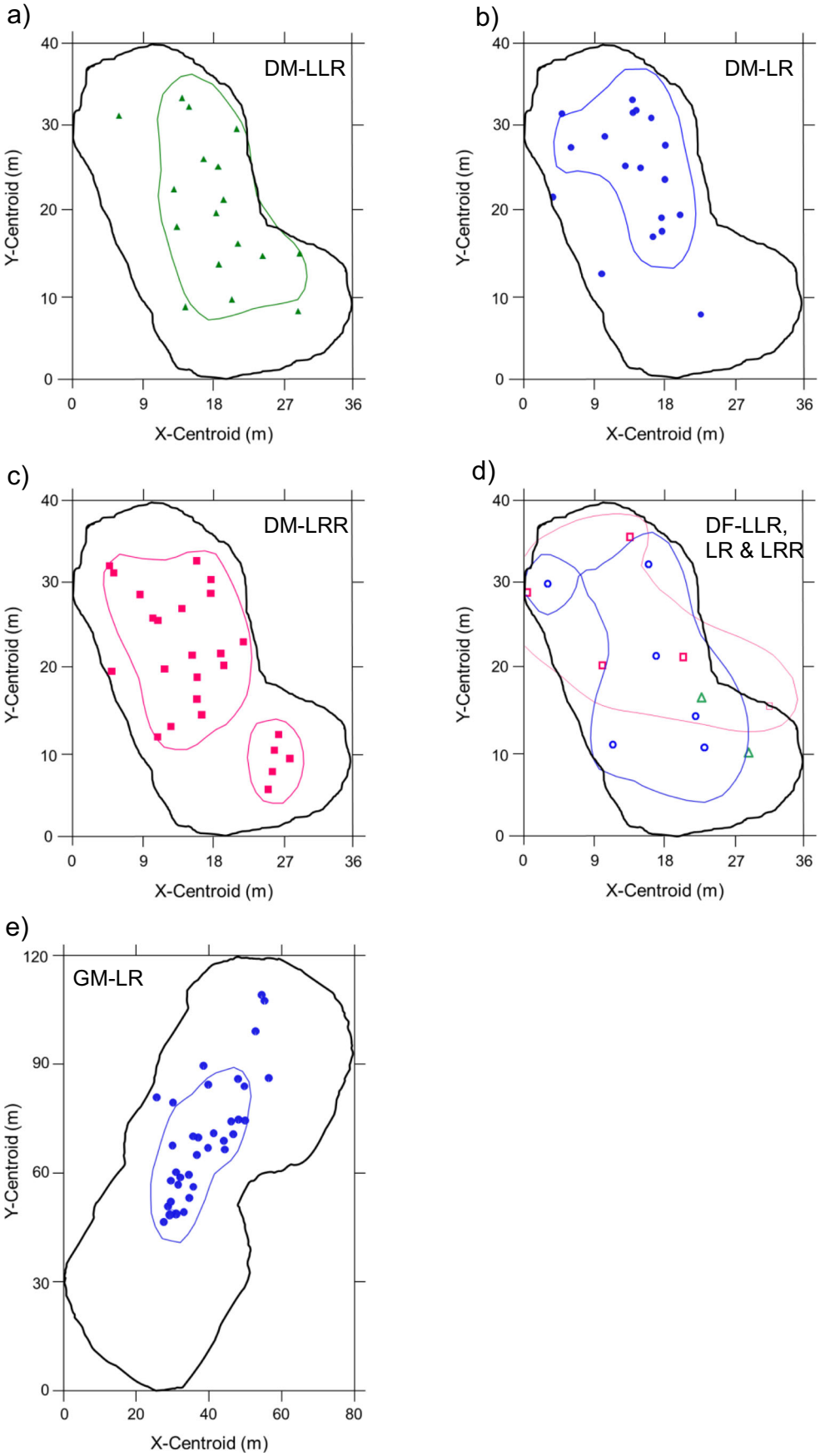


Fig. 5 Distribution of centroids for males (**a**, **b**) and females (**c**, **d**) of *P. lessonae* (*LL*) and *P. esculentus* (*LR*) in Kloten. *Green lines* indicate 68 % kernel estimates of the distributions

the population with the highest male/female ratio (Table 1) than in the Kloten and Döbern populations with the lower sex ratio skew. For several anuran species, it has been shown that with increasing competition males tend to shift their mating strategy from stationary calling to active searching for females (reviewed by Wells 2007). This may, in part, explain why all three distance measures (DNN, DBO and DTC) were higher in Genarp than in Kloten and Döbern.

Body size and condition Significant effects of body size and condition were observed only for distance to the nearest neighbor (DNN) in Kloten (Table 5). After correcting for genotype, DNN decreased with average size and condition of the respective individuals (AV_SVL, AV_BCI). This means that large males in good condition kept closer to each other, but the distance increased with the size difference between them (DIFF_SVL). Larger males—which, on average, are also older (Blankenhorn 1974; Embrechts and Reyer 2012)—may be more inclined to compete, thus leading to shorter distances between them. They also may be more successful in keeping smaller males at bay, either through direct physical combat as, for instance, observed in toads (*Bufo bufo*) by Davies and Halliday (1979) or as a consequence of small males avoiding large males on the basis of their size, activity, call characteristics, and other features (Wells 1977). In some species, this can lead to larger males obtaining higher-quality territories than


a)
DM-LLR
b)
DM-LR
c)
DM-LRR
d)
DF-LLR,
LR & LRR
e)
GM-LR
Y-Centroid (m)
X-Centroid (m)
0
9
18
27
36
10
20
30
40
60
80
90
120

◀ **Fig. 6** Distribution of centroids for males (**a**–**c**, **e**) and females (**d**) in Döbern (**a**–**d**) and Genarp (**e**). *Symbols* and *colors* code for different genotypes: *green triangles* LLR, *blue circles* LR, *red squares* LRR. *Filled symbols* indicate males, *open symbols* indicate females. *Matching colored lines* indicate 68 % kernel estimates of the distributions. In the case of LLR females from Döbern (**d**), low sample size prevented the calculation of a kernel estimate. In Genarp (**e**), only one female data point was available, and therefore only males are shown

small males as Howard (1978), for instance, has found for bullfrogs (*Rana catesbeiana*).

The effect of differences in body condition on DNN was less clear (absent in Kloten, negative in Döbern; Table 5). This may be due to smaller variation in BCI (0.13) than in SVL (0.18).

Genotype Genotype had no significant effect on any of the measured spatial and movement behaviors in either the L–E population of Kloten or in the all-hybrid E–E population of Döbern (Table 3). (Due to a small sample size, we could not test for differences between genotypes in Genarp.) This contrasts with some previous studies that did find genotype-related behavioral differences, including in movement and site fidelity during the mating season. *Pelophylax lessonae* males tend to roam around in choruses to intercept and clasp females, whereas *P. esculentus* seems to be more stationary, territorial, and aggressive, thus resembling *P. ridibundus* (for more details and the relevant literature, see "Introduction"). Although we did not quantify aggressive behavior in our study, indirect evidence from the two populations with almost identical DNN supports this finding. In the all-hybrid pond in Döbern, we frequently observed intense fighting over females and aggressive dislocation of amplexing males by other males with the result that several females incurred skin wounds. In one case, we found a recently perished female with parts of her intestines and ripe ovaries emerging from the body. In contrast, in Kloten, where 87 % of the males were *P. lessonae* (Table 1), we observed only few physical aggressive encounters between males and no obvious amplexus wounds.

Some studies have found behavioral differences between genotypes on an even finer scale, namely, in line with a genome dosage effect, i.e., with the ratio of L/R genomes decreasing in the order LL<LLR<LR<LRR<RR. This ratio was, for instance, found to fully explain differences in five parameters of male advertisement calls (Hoffmann and Reyer 2013), and it can partly explain why habitat preferences of hybrids are intermediate between those of the two parental species (Günther 1990; Plenet et al. 2000; Pagano et al. 2001; Holenweg Peter et al. 2002; Plötner 2005; Jakob et al. 2010).

Male mating success in relation to spatial behavior

The previously documented existence of phenotype- and genotype-related behavioral differences and their effects on male mating success raises the question why they were not detected in our study. Movement patterns and spatial distribution did not differ between genotypes, and overall, the probability of being observed in amplexus was not related to body size and condition, nor did it differ significantly from what was expected from the genotype proportions among males in the population.

One possible explanation is that body size, movement, and spatial arrangement of males alone might not be good indicators for their mating success. This has been shown in several studies, e.g., one on *Bufo americanus* (Gatz 1981) and one on

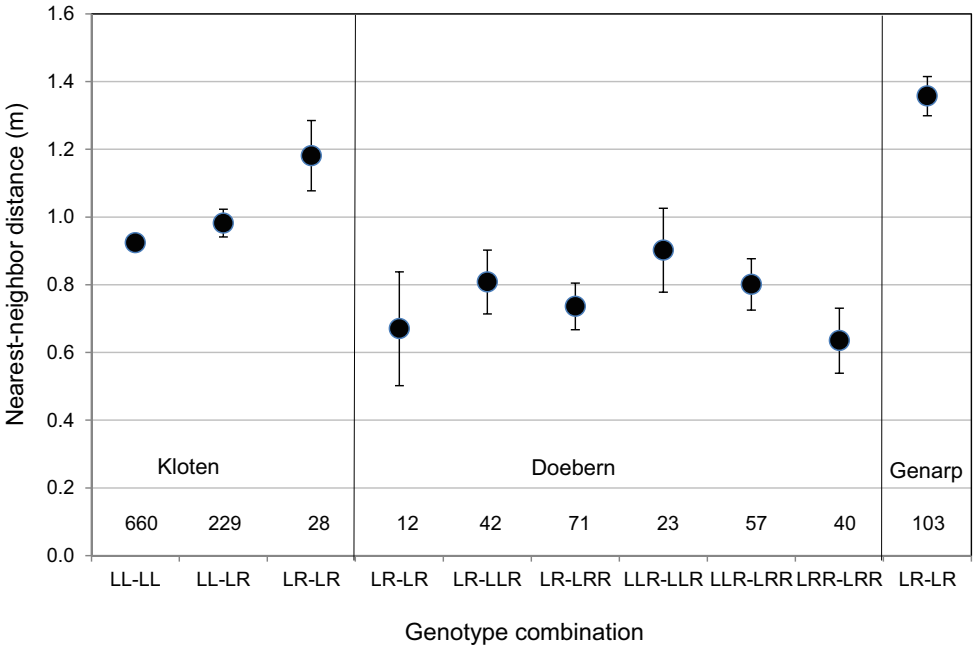


Fig. 7 Nearest-neighbor distances (means ± SE) between different pairs of genotypes in the three study populations: Kloten, Döbern, and Genarp

Table 5 Results from multiple regression (Genarp) and GLM analyses (Kloten and Döbern) on relationships between male nearest-neighbor distances and body size (SVL, average and differences), body condition (BCI, average and differences), and genotype of neighbors. Due to the almost complete lack of neighborhoods other than diploid hybrids (LR–LR) in Genarp, the genotype effect could not be tested for this population (significant results are in italics)

Source	Kloten				Döbern				Genarp			
	df	Coefficient	*F*-ratio	*p*	df	Coefficient	*F*-ratio	*p*	df	Coefficient	*t*	*p*
Genotypes	2		2.849	0.058	5		1.273	0.276	–	–	–	–
AV_SVL	1	−0.029	12.632	*<0.001*	1	−0.013	1.432	0.233	1	0.017	0.965	0.337
AV_BCI	1	−0.453	6.042	*0.014*	1	−1.016	2.276	0.133	1	0.155	0.102	0.919
DIFF_SVL	1	0.015	8.674	*0.003*	1	0.007	0.624	0.431	1	−0.018	−1.268	0.208
DIFF_BCI	1	0.074	0.292	0.589	1	−1.121	4.420	*0.037*	1	0.423	0.427	0.670
Error	910				235				98			

P. lessonae from an L–E system in France (Lengagne and Joly 2010). Our failure to find higher success in males that are larger and/or better located in places that are more attractive for females may be due to the fact that their advantage can be attenuated or balanced through alternative mating tactics of the inferior males. These include, for instance, searching in more peripheral areas or keeping as "satellites" close to large attractive males to intercept females on their way to those males (Wells 1977; Arak 1983; Forester and Thompson 1998).

This illustrates a problem that can arise when spatial data are used to infer the underlying network of affiliations. Although it is a plausible, and often empirically supported, assumption that sexual, aggressive, and cooperative interactions are more frequent between close neighbors than between distant ones (Oh and Badyaev 2010), this may not always be true when alternative mating tactics exist. Also, at very high densities and clumped female occurrence, male spatial structure can temporarily break down to a scramble competition, even in populations that show resource defense polygyny or lekking behavior at lower densities (e.g., Grant et al. 1995; Byrne and Roberts 2004). Moreover, there is the problem of the right observation length: if the time window is too small, important connections may not be captured; if it is too large, meaningless links may emerge (Psorakis et al. 2012). Both problems existed in our study. Especially in Kloten, the pond with the highest density, we regularly observed that males left their usual positions for a while to congregate in dense choruses in a small area, where females had arrived, and vigorously fought over access to them (GAT, unpublished data). With no behavioral observations at night, and at least 5 h between them during daytime, the spatial and movement data that we collected (i.e., the adjacency matrix) may not have been good proxies for the interactions (i.e., the incidence matrix) that led to mating success. Other studies have shown that it is this attendance of choruses, rather than body size, age, or other factors, that determines male mating success. (e.g., Friedl and Klump 2005).

There are, however, also biological reasons for not finding an obvious relationship between genotype-related spatial behavior and male mating success. These have to do with the very specific water frog breeding system. As outlined in the "Introduction", both theoretical models and empirical studies have shown that in all-hybrid E–E systems, preference for a certain genotype cannot—and apparently has not—evolved (Som and Reyer 2006; Rondinelli 2006). Where, however, female preferences do not exist and mating is apparently random, males of all genotypes are equally competent competitors in the sense that they have equal chances to mate. This has been demonstrated in a natural population by Günther and Plötner (1990). Hence, in all-hybrid E–E systems, all male genotypes are likely to profit from the same mating strategies, and it is not surprising that in the two populations of this type (Döbern and Genarp) movement patterns (Table 4) and distances to the nearest neighbors did not differ between genotypes (Table 5; Fig. 7).

In the L–E system, on the other hand, where hybrid females should and do prefer males of the parental species, males of both genotypes should seek the vicinity of the respective other genotype: *P. esculentus* should seek *P. lessonae* neighbors to benefit from their experimentally demonstrated higher attractiveness to females, and *P. lessonae* should stay close to *P. esculentus* males because their lower attractiveness to females makes them less serious competitors. Therefore, we would expect nearest-neighbor distances between genotypes (LL–LR) to be shorter than those within genotypes (LL–LL, LR–LR). There is, indeed, a tendency for this to be true when LL–LR distances are compared to LR–LR distances; the expected higher LL–LL distance, however, was not found (Table 5; Fig. 7). A mechanistic reason could be that with almost seven times as many LL as LR (Table 1), the LL were so densely packed that spacing out was not possible. A biological reason might be that searching for and maintaining the preferred spatial context is often costly in terms of time and energy expenditure. If these costs are equal to all individuals, relatively unattractive males, which benefit the most in terms

of mating success (here LR), should be more inclined to invest into creating favorable social environments compared to more attractive males (here LL) (Oh and Badyaev 2010).

General conclusions

The general message from our results is as follows: Although there is a close and often demonstrated link between mating systems and the spatial distribution of resources, mating partners, and competitors, as proposed by the ecological framework model (Orians 1969; Emlen and Oring 1977), not all differences between mating systems come along with differences in spatial distribution and movement patterns. In our water frogs, movement patterns and spatial distribution were similar for all genotypes in both the E–E and the L–E systems. Differences in space use are only to be expected when there is a conflict between the involved parties as, for instance, between territory holders and individuals pursuing alternative mating strategies. In birds, for instance, unpaired males may try to enter the territories of paired males for obtaining extra-pair copulations, whereas the territory holders will try to keep them out or guard their females when they leave for common feeding grounds (Reyer et al. 1997; Westneat and Mays 2005; Cockburn et al. 2009; Mayer and Pasinelli 2013). In the studied water frogs, however, there is no conflict in either population system. In the E–E system, no preferred neighbor type exists because both theoretical models and empirical data allude to random mating as the best strategy (Som and Reyer 2006; Rondinelli 2006). In the L–E system, with predicted and demonstrated female choice (Abt and Reyer 1993; Roesli and Reyer 2000; Engeler and Reyer 2001; Som et al. 2000), both hybrid and parental males should prefer a neighbor of the respective other genotype, i.e., LL should seek the vicinity of the, for females, less attractive LR, and LR should seek the vicinity of the more attractive LL. Such congruence about the preferred neighbor is not restricted to this, admittedly, unusual frog system. It has, for instance, also been found between territory holders and their satellites (e.g., Taborsky 1994; Ortega and Arita 2002).

Although our results are in line with those expected for the E–E and L–E breeding systems, it should be kept in mind that in natural populations, experimentally demonstrated preferences can be mitigated by several factors. These include, among other things, lack of preferred males and intense male–male competition (Bergen et al. 1997), female encounters with less attractive males while approaching a male they actually prefer (Gerhardt et al. 1994), density- and age-related differences in male sexual activity (Blankenhorn 1974; Woolbright et al. 1990), and high levels of background noise in dense choruses (Gerhardt and Klump 1988; Johnstone and Earn 1999), which reduces female abilities to discriminate between competing males (Richardson and Lengagne 2010). As a result, mating systems and reproductive behavior vary considerably with environmental conditions, density, demography, and their changes in space and time (Kokko and Rankin 2006; Wells 2007). Hence, our above interpretation of the possible links between the distribution, movements, and mating success of different genotypes in natural populations should be taken with a grain of salt and lead to further hypotheses that can be tested experimentally. One experiment that investigates the effects of different genotype and sex ratios on movement and spatial distribution is presently underway.

Acknowledgments We greatly thank Irene Völlmy for her help in the field in Döbern and Genarp, Sandra Röthlisberger for her excellent lab work, and two anonymous reviewers for their helpful comments on the first version of the manuscript. Special thanks go to the pond owners, who kindly allowed us to roam in and around their ponds over several weeks—and sometimes even treated us with meals and drinks. The study was funded through the University of Zürich and a grant by the Swiss National Science Foundation to H-UR (no. 3100A0-120225/1).

Ethical standards The experiments comply with the current laws of Sweden, Germany, and Switzerland.

References

Abt Tietje GJ (2003) Pond use, patterns of reproduction and juvenile recruitment in a mixed waterfrog population. Ph.D. thesis, Universität Zürich

Abt G, Reyer H-U (1993) Mate choice and fitness in a hybrid frog: *Rana esculenta* females prefer *Rana lessonae* males over their own. Behav Ecol Sociobiol 32:221–228

Alves MJ, Coelho MM, Collares-Pereira MJ (2001) Evolution in action through hybridisation and polyploidy in an Iberian freshwater fish: a genetic review. Genetica 111:375–385

Arak A (1983) Male–male competition and mate choice in anuran amphibians. In: Bateson P (ed) Mate choice. Cambridge University Press, Cambridge, pp 181–210

Arioli M (2007) Reproductive patterns and population genetics in pure hybridogenetic water frog populations of *Rana esculenta*. Ph.D. thesis, Universität Zürich

Arioli M, Jakob C, Reyer HU (2010) Genetic diversity in water frog hybrids (*Pelophylax esculentus*) varies with population structure and geographic location. Mol Ecol 19:1814–1828

Arnold ML (1997) Natural hybridization and evolution. Oxford University Press

Bergen K, Semlitsch RD, Reyer HU (1997) Hybrid female matings are directly related to the availability of *Rana lessonae* and *Rana esculenta* males in experimental populations. Copeia 1997:275–283

Blankenhorn H (1974) Soziale Organisation einer Mischpopulation von *Rana lessonae* Camerano und *Rana esculanta* Linnaeus. Ph.D. thesis, Universität Zürich

Bullini L (1994) Origin and evolution of animal hybrid species. Trends Ecol Evol 9:422–426

Byrne PG, Roberts GA (2004) Intrasexual selection and group spawning in quacking frogs (*Crinia georgiana*). Behav Ecol 15:872–882

Caughley G (1980) Analysis of vertebrate populations. Wiley, London

Christiansen DG (2005) A microsatellite-based method for genotyping diploid and triploid water frogs of the *Rana esculenta* hybrid complex. Mol Ecol Notes 5:190–193

Christiansen DG (2009) Gamete types, sex determination and stable equilibria of all-hybrid populations of diploid and triploid edible frogs (*Pelophylax esculentus*). BMC Evol Biol 9:135

Christiansen DG, Reyer HU (2009) From clonal to sexual hybrids: genetic recombination via triploids in all-hybrid populations of water frogs. Evolution 63:1754–1768

Christiansen DG, Fog K, Pedersen BV, Boomsma JJ (2005) Reproduction and hybrid load in all-hybrid populations of *Rana esculenta* water frogs in Denmark. Evolution 59:1348–1361

Cockburn A, Dalziell AH, Blackmore CJ, Double MC, Kokko H, Osmond HL, Beck NR, Head ML, Wells K (2009) Superb fairy-wren males aggregate into hidden leks to solicit extragroup fertilizations before dawn. Behav Ecol 20:501–510

Cody ML (1985) Habitat selection in birds. Academic, London

Davies NB, Halliday TR (1979) Competitive mate searching in male common toads, *Bufo bufo*. Anim Behav 27:1253–1285

Dawley RMBJP (1989) Evolution and ecology of unisexual vertebrates. The New York State Museum, Bulletin 466, Albany

Ebendal T (1979) Distribution, morphology and taxonomy of the Swedish green frogs (*Rana esculenta* complex). Mitt Zool Mus Berlin 55:143–152

Ebendal T, Uzzell T (1982) Ploidy and immunological distance in Swedish water frogs (*Rana esculenta* complex). Amphibia-Reptilia 3:125–133

Embrechts E, Reyer HU (2012) Age and size of hybrid water frogs: the role of genotype and ecology. Herpetologica 68:468–481

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197:215–223

Engeler B, Reyer H-U (2001) Choosy females and indiscriminate males: mate choice in mixed populations of sexual and hybridogenetic water frogs (*Rana lessonae, Rana esculenta*). Behav Ecol 12:600–606

Forester DC, Thompson KJ (1998) Gauntlet behaviour as a male sexual tactic in the American toad (Amphibia: Bufonidae). Behaviour 135: 99–119

Friedl TWP, Klump GM (2005) Sexual selection in the lek-breeding European treefrog: body size, chorus attendance, random mating and good genes. Anim Behav 70:1141–1154

Gatz AJ Jr (1981) Non-random mating by size in American toads, *Bufo americanus*. Anim Behav 29:1004–1012

Gerhardt HC, Klump GM (1988) Masking of acoustic signals by the chorus background-noise in the green tree frog: a limitation on mate choice. Anim Behav 36:1247–1249

Gerhardt HC, Dyson ML, Tanner SD, Murphy CG (1994) Female treefrogs do not avoid heterospecific calls as they approach conspecific calls—implications for mechanisms of mate choice. Anim Behav 47:1323–1332

Glaettli M, Pescatore L, Goudet J (2006) Proximity-dependent pollen performance in *Silene vulgaris*. Ann Bot 98:431–437

Graf J-D, Polls Pelaz M (1989) Evolutionary genetics of the *Rana esculenta* complex. In: Dawley R, Bogart JP (eds) Evolution and ecology of unisexual vertebrates. New York State Museum, New York, pp 289–301

Grant JWA, Bryant MJ, Soos CE (1995) Operational sex ratio, mediated by synchrony of female arrival, alters the variance of male mating success in Japanese medaka. Anim Behav 49:367–375

Grosberg RK (1987) Limited dispersal and proximity-dependent mating success in the colonial Ascidian *Botryllus schlosseri*. Evolution 41: 372–384

Günther R (1990) Die Wasserfrösche Europas. A. Ziemsen Verlag, Wittenberg Lutherstadt

Günther R, Plötner J (1990) Mating pattern in pure hybrid populations of water frogs, *Rana* kl. *esculenta* (Anura, Ranidae). Alytes 8:90–98

Günther R, Uzzell T, Berger L (1979) Inheritance patterns in triploid *Rana* "*esculenta*" (Amphibia, Salientia). Mitt Zool Mus Berlin 55:35–57

Hellriegel B, Reyer HU (2000) Factors influencing the composition of mixed populations of a hemiclonal hybrid and its sexual host. J Evol Biol 13:906–918

Heym W-D (1974) Studien zur Verbreitung, Ökologie und Ethologie der Grünfrösche in der Mittleren und Nördlichen Niederlausitz. Mitt Zool Mus Berlin 50:263–285

Hoffmann A, Reyer HU (2013) Genomic effects on advertisement call structure in diploid and triploid hybrid waterfrogs (Anura, *Pelophylax esculentus* kl.). BMC Ecol 13:47

Holenweg Peter AK, Reyer HU, Abt Tietje G (2002) Species and sex ratio differences in mixed populations of hybridogenetic water frogs: the influence of pond features. Ecoscience 9:1–11

Howard RD (1978) The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution 32:850–871

Jakob EM, Marshall SD, Uetz GW (1996) Estimating fitness: a comparison of body condition indices. Oikos 77:61–67

Jakob C, Arioli M, Reyer H-U (2010) Ploidy composition in all-hybrid frog populations in relation to ecological conditions. Evol Ecol Res 12:633–652

Johnstone RA, Earn DJD (1999) Imperfect female choice and male mating skew on leks of different sizes. Behav Ecol Sociobiol 45: 277–281

Kierzkowski P, Pasko L, Rybacki M, Socha M, Ogielska M (2011) Genome dosage effect and hybrid morphology—the case of the hybridogenetic water frogs of the *Pelophylax esculentus* complex. Ann Zool Fenn 48:56–66

Kokko H, Rankin DJ (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philos Trans R Soc B 361: 319–334

Krebs CJ (2009) Ecology: the experimental analysis of distribution and abundance, 6th edn. Pearson Benjamin Cummings, San Francisco

Kuhn B, Schneider H (1984) Mating and territorial calls of *Rana ridibunda* and their temperature-dependent variability. Zool Anz 212:273–305

Kyriakopoulou-Sklavounou P, Loumbourdis N (1990) Contribution to the resproductive biology of *Rana ridibunda* Pallas (Anura, Ranidae). Amphibia-Reptilia 11:23–30

Lengagne T, Joly P (2010) Paternity control for externally fertilised eggs: behavioural mechanisms in the waterfrog species complex. Behav Ecol Sociobiol 64:1179–1186

Lengagne T, Grolet O, Joly P (2006) Male mating speed promote hybridization in the *Rana lessonae-Rana esculenta* waterfrog system. Behav Ecol Sociobiol 60:123–130

Lengagne T, Plenet S, Joly P (2008) Breeding behaviour and hybridization: variation in male chorusing behaviour promotes mating among taxa in waterfrogs. Anim Behav 75:443–450

Mayer C, Pasinelli G (2013) New support for an old hypothesis: density affects extra-pair paternity. Ecol Evol 3:694–705

Mitchell MA (2009) Anesthetic considerations for amphibians. J Exotic Pet Med 18:40–49

Oh KP, Badyaev AV (2010) Structure of social networks in a passerine bird: consequences for sexual selection and the evolution of mating strategies. Am Nat 176:E80–E89

Orians GH (1969) On the evolution of mating systems on birds and mammals. Am Nat 103:589–603

Orians GH, Wittenberger JF (1991) Spatial and temporal scales in habitat selection. Am Nat 137:29–49

Ortega J, Arita HT (2002) Subordinate males in harem groups of Jamaican fruit-eating bats (*Artibeus jamaicensis*): satellites or sneaks? Ethology 108:1077–1091

Pagano A, Joly P, Plenet S, Lehman A, Grolet O (2001) Breeding habitat partitioning in the *Rana esculenta* complex: the intermediate niche hypothesis supported. Ecoscience 8:294–300

Pfennig KS (2007) Facultative mate choice drives adaptive hybridization. Science 318:965–967

Plenet S, Hervant F, Joly P (2000) Ecology of the hybridogenetic *Rana esculenta* complex: differential oxygen requirements of tadpoles. Evol Ecol 14:13–23

Plötner J (2005) Die westpaläarktischen Wasserfrösche. Laurenti, Bielefeld

Plötner J, Klinghardt M (1992) Investigations on the genetic structure and the morphometry of a pure hybrid population of *Rana esculenta* (Anura, Ranidae) in North Germany. Zool Anz 229:163–184

Plötner J, Becker C, Plötner K (1994) Morphometric and DNA investigations into European water frogs (*Rana kl.esculenta* Synklepton (Anura, Ranidae) from different population systems. Z Zool Syst Evol 32:193–210

Psorakis I, Roberts SJ, Rezek I, Sheldon BC (2012) Inferring social network structure in ecological systems from spatio-temporal data streams. J R Soc Interface 9:1–10

Reyer HU (2008) Mating with the wrong species can be right. Trends Ecol Evol 23:289–292

Reyer H-U, Bollmann K, Schläpfer AR, Schymainda A, Klecack G (1997) Ecological determinants of extrapair fertilizations and egg dumping in Alpine water pipits (*Anthus spinoletta*). Behav Ecol 8: 534–543

Reyer H-U, Frei G, Som C (1999) Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. Proc R Soc Lond B 266:2101–2107

Richardson C, Lengagne T (2010) Multiple signals and male spacing affect female preferences at cocktail parties in treefrogs. Proc R Soc Lond B277

Roesli M, Reyer HU (2000) Male vocalization and female choice in the hybridogenetic *Rana lessonae/Rana esculenta* complex. Anim Behav 60:745–755

Rondinelli B (2006) Female choice in all-hybrid populations of *Rana esculenta*. M.Sc. thesis, Universität Zürich, Zurich

Schultz RJ (1969) Hybridization, unisexuality and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Am Nat 103:605–619

Som C, Reyer HU (2006) Demography and evolution of pure hybridogenetic frog (*Rana esculenta*) populations. Evol Ecol Res 8:1235–1248

Som C, Anholt BR, Reyer HU (2000) The effect of assortative mating on the coexistence of a hybridogenetic waterfrog and its sexual host. Am Nat 156:34–46

Sutherland WJ (1996) From individual behaviour to population ecology. Oxford University Press, Oxford

Taborsky M (1994) Sneakers, satellites, and helpers—parasitic and cooperative behavior in fish reproduction. Adv Stud Behav 23:1–100

Team QD (2013) QGIS geographic information system. Open Source Geospatial Foundation Project, http://qgis.osgeo.org

Tunner HG (1974) Die klonale Struktur einer Wasserfroschpopulation. Z Zool Syst Evol 12:309–314

Tunner HG (1976) Aggressives Verhalten bei *Rana ridibunda*, *Rana lessonae* und der hybriden *Rana esculenta*. Zool Anz 200:386–390

Tunner HG (2000) Evidence for genomic imprinting in unisexual triploid hybrid frogs. Amphibia-Reptilia 21:135–141

Tunner HG, Heppich-Tunner S (1991) Genome exclusion and two strategies of chromosome duplication in oogenesis of a hybrid frog. Naturwissenschaften 78:32–34

Uzzell T, Berger L (1975) Electrophoretic phenotypes of *Rana ridibunda, Rana lessonae*, and their hybridogenetic associate, *Rana esculenta*. P Natl Acad Sci USA 127:13–24

Uzzell T, Hotz H (1979) Electrophoretic and morphological evidence for two forms of green frogs (*Rana esculenta* Complex) in peninsular Italy (Amphibia, Salientia). Mitt Zool Mus Berlin 55:13–27

Wahl M (1969) Untersuchungen zur Bio-Akustik des Wasserfrosches *Rana esculenta* (L.). Oecologia 3:14–55

Weidenberg K (1999) Vergleichende Untersuchugnen zum Paarungs- und Territorialverhalten von *Rana ridibunda*- und *Rana kl. esculenta*-Männchen. M.Sc. thesis, Humboldt-Universität, Berlin

Wells KD (1977) The social behaviour of anuran amphibians. Anim Behav 25:666–693

Wells KD (2007) The ecology and behavior of amphibians. The University of Chicago Press, Chicago

Westneat DF, Mays HL (2005) Tests of spatial and temporal factors influencing extra-pair paternity in red-winged blackbirds. Mol Ecol 14:2155–2167

Woolbright LL, Greene EJ, Rapp GC (1990) Density-dependent mate searching strategies of male woodfrogs. Anim Behav 40:135–142

Zalesna A, Choleva L, Ogielska M, Rabova M, Marec F, Rab P (2011) Evidence for integrity of parental genomes in the diploid hybridogenetic water frog *Pelophylax esculentus* by genomic in situ hybridization. Cytogenet Genome Res 134:206–212

Zippin C (1958) The removal method of population estimation. J Wildl Manag 22:82–90